ACUTE PHASE RESPONSE PROFILES AS INDICATORS OF DISEASE SEVERITY, RECOVERY OR VACCINE EFFICACY IN BOVIDS INFECTED WITH *PASTEURELLA MULTOCIDA*

Hodgson JC
Moredun Research Institute, Bacteriology Division, Pentlands Science Park, Bush Loan, Penicuik, UK.
Email:hodgc@mri.sari.ac.uk

The potential diagnostic value of acute phase proteins as markers of inflammation and infectious disease may be assessed by measuring changes in their serum concentrations during experimental infectious challenge studies. Plasma concentrations of acute phase proteins change by at least 25% during inflammation and are useful markers with which to determine the progression of disease with time and to characterise initial host responses. Hp is particularly useful as a sensitive marker of bacterial infection, increasing between 10- and 100-fold after challenge. Such studies provide information also on the function and possible therapeutic role of acute phase proteins. The role of the acute phase response in pneumonic and systemic infectious disease in ruminants caused by the Gram-negative bacterium *Pasteurella multocida* is the subject of the following review. *P. multocida* serotype A:3 is a major cause of pneumonic pasteurellosis in cattle, the incidence of which is rising steadily in the UK whereas serotype B:2 causes haemorrhagic septicaemia (HS) in South and Southeast Asia, resulting in severe economic losses amongst livestock through morbidity and mortality, particularly in buffaloes (*Bubalus bubalis*).

Disease severity

Pneumonic pasteurellosis

Pneumonic pasteurellosis in young ruminants due to *P. multocida* serotype A is an infectious disease of high economic and welfare importance, but the mechanism of disease is poorly defined and the prevention and treatment of disease remains inadequate. The involvement of acute phase proteins in the pathogenesis and resolution of disease has been studied in calves challenged intratracheally with about 10^9 colony forming units (cfu) *P. multocida* A:3 given either in low (60ml) or high (300ml) volumes of suspension. All experimental protocols were approved by the Moredun Research Institute Animal Experiments Committee, authorised under the Animals (Scientific Procedures) Act 1986 and access to veterinary care was available at all times. Concentrations in jugular vein blood samples of acute phase proteins haptoglobin (Hp, using purified bovine Hp as a standard), α_1-acid glycoprotein (AGP, by radial immunodiffusion kit, Saikin Kagaku Institute, Japan) and serum amyloid A (SAA, by ELISA, Tridelta Phase range, Greystones, Ireland) against time were summarised using the area under the curve (AUC) adjusted to individual time-zero baseline values and analysed using REML. Plasma Hp concentrations increased linearly to a mean value of about 1g/l, significantly greater ($P<0.05$) than the mean of the pre-infection samples, falling gradually thereafter. Increases in the concentration α_1-AGP were more gradual than those observed for SAA or Hp and maintained for longer, reaching a peak mean concentration of 435 mg/l at 48 h post-infection (p.i.) declining thereafter with a slight recovery between 72–96 h p.i. Concentrations of SAA increased rapidly between 5 and 23 h p.i., remaining elevated (approximately 400 –600 mg/l) until falling dramatically to about 5 mg/l between 72 and 96 h p.i.

All treatments induced clinical signs characteristic of bovine pneumonic pasteurellosis as observed in natural cases of disease, including moderate depression, pyrexia, laboured breathing, mild nasal discharge, and anorexia. In addition, the gross pathological and microscopic changes observed were similar to those reported both for experimental induction and field cases of bovine pneumonic pasteurellosis associated with *P. multocida*. The acute phase protein results indicated that, of the two treatment variables used (dose and volume), volume was the more influential factor inducing pneumonic disease. It was interesting that animals given the high volume challenge exhibited higher ($P<0.05$) peak responses in Hp. The high volume challenge probably affected a greater area of lung, especially if an initially slow response in Hp production allowed more time for the bacteria to proliferate. Examination of α_1-AGP concentrations for all treatments indicated that the high volume challenge gave rise to a greater increase from resting level than the low volume challenge. Similar correlation of α_1-AGP with severity of disease has been reported for other disorders aside from pneumonic pasteurellosis, and in previous work examining natural acute versus chronic inflammation in cattle. However, in the present work there was no correlation between acute phase protein concentrations and lung bacterial clearance.

Systemic disease

Haemorrhagic septicaemia in buffalo, a form of endotoxin shock, is characterised by a short clinical course (hours) and signs may include severe depression, pyrexia, submandibular oedema, dyspnoea followed by recumbency and death. There is no reliable long-acting vaccine and the pathogenic mechanisms are poorly defined. To investigate the role of the acute phase response during the disease, changes in acute phase protein concentrations were monitored during development of haemorrhagic

septicaemia in buffalo after intranasal infection with a representative strain of *P. multocida* B:2. Mean serum Hp concentrations in infected animals rose by 24h p.i. and the mean increase was significantly higher ($P<0.05$) than that in control (non-infected) animals, reaching a peak of 2.5 ± 0.6 mg/ml at 72h p.i. Serum AGP concentrations increased significantly ($P<0.05$) in surviving animals to about 300 μg/ml between 48h and 120h after infection but showed no change from a mean baseline concentration of 80 ± 6.6 μg/ml in control animals or in infected animals that succumbed to infection

Recovery phase

Studies of the pathogenesis of haemorrhagic septicaemia indicated that surviving animals went on to develop greater levels of Hp and α_1-AGP. This was perhaps instrumental in restricting bacteraemia to levels below those that trigger endotoxaemia as acute phase proteins have been shown to enhance protection against bacterial infections possibly by modifying the inflammatory responses through effects on cell trafficking and mediator release.

Responses after vaccination

In efficacy studies in calves using an attenuated live AroA mutant of *P. multocida* B:2 as a potential vaccine against haemorrhagic septicaemia, SAA concentrations after intramuscular (i.m.) or intranasal (i.n.) vaccination were compared. Different patterns of response were observed between i.m. and i.n. vaccinated calves: concentrations increased significantly ($P < 0.05$) in i.m. vaccinated calves but showed no significant changes in i.n. vaccinated calves. The SAA concentrations declined in i.m. vaccinated calves after 24 h but increased again significantly ($P < 0.001$) in response to a second vaccination. No significant changes due to a second vaccination were noted for i.n. vaccinated calves.

The concentrations of SAA in individual calves varied considerably, and the means before challenge were highest in the control calves, followed by i.m. vaccinated and then by i.n. vaccinated calves. The mean SAA concentration in i.n. vaccinated animals increased 13-fold by 10 h after challenge but by only 66 and 11% in i.m. vaccinated and control calves, respectively, and this difference between i.n. vaccinated calves and the others was statistically significant ($P < 0.01$). At 23 h p.i., SAA values exceeded 158 μg/ml in the two calves, one each from the i.n. vaccinated and control groups, that had survived, whereas the maximum increase observed for calves in the i.m. vaccinated group was only threefold (to 37 ± 15.6 μg/ml). High SAA concentrations took 6 to 8 days to return to prechallenge levels in the surviving calves. One calf from each of the otherwise vulnerable i.n. vaccinated and control groups did not develop disease. The reasons why not all animals succumb to infection are not clear, although it may that a gradual but sustained rise in acute phase proteins may play a part in helping to control disease. Thus, the large increase in SAA concentrations after challenge observed in these particular calves may have contributed to their survival. On the other hand, changes in the concentrations of SAA may also be used to indicate the progress and severity of infection. In the present work, the large increase in SAA concentrations observed in i.n. vaccinated animals upon challenge, compared with the moderate response of the i.m. group, may well have reflected a less well-developed immune protection in the former group. However, there was no clear indication that a higher SAA level prechallenge might represent a marker for subsequent survival, as an i.n. vaccinated calf that subsequently survived challenge had a low SAA level of 4.3 μg/ml while a control calf that survived had a high level of 128 μg/ml at the point of challenge. The acute phase response to the initial i.m. vaccination paralleled the clinical response (increased rectal temperature and overall clinical score), indicating that the attenuated bacteria persisted long enough to promote an inflammatory response.

Other work showed strong evidence ($P<0.001$) that calves that had high levels of Hp or α_1-AGP before challenge tended to have high levels of Hp or α_1-AGP, respectively, throughout the first five days p.i.. A rapid rise in plasma SAA concentrations for calves immunised via intratracheal instillation of killed *P. multocida* was similar to that reported for cattle challenged intratracheally in other work with live *Mannheimia haemolytica* but less than the response observed following live *P. multocida* challenge. The lack of any statistically significant differences in Hp concentrations between immunised and control animals contrasted markedly to that following a live challenge of *P. multocida* when Hp concentrations increased linearly to a peak at 5 h p.c of 1 g l^{-1}.

Conclusions

Concentrations of the acute phase proteins Hp, SAA and α_1-AGP increase following experimental infectious challenge with *P. multocida* A:3, suggesting a role for these proteins as markers of the onset and progress of bovine pneumonic pasteurellosis or, in the case of α_1-AGP, as contributors to controlling bacterial infection. Similar responses are noted in experimental challenge studies in cattle and buffalo infected with *P. multocida* B:2. Future detailed studies of host acute phase response together with host innate immune responses *in vivo* and *in vitro* will help extend our understanding of the pathogenic mechanisms involved and how to combat disease caused by *P. multocida*.

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